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0014-4754/89/060521-06$1.50 + 0.20/0

Expression of the genes of interferons and other cytokines in normal and diseased tissues of man

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Summary. Specific interferon genes are transcribed at low levels in the spleen, liver, and peripheral blood leukocytes of normal individuals in the apparent absence of virus infection while other interferon genes remain unexpressed in the same tissues. In contrast, the genes of cytokines such as IL-1, IL-6 and TNF are expressed at relatively high levels in the organs of normal individuals. The level of expression of the IL-1, IL-6 and TNF genes is markedly reduced in the livers of patients with autoimmune liver disease compared to the level of expression in the liver of normal individuals, whereas the expression of interferon genes is similar in both normal and diseased liver, suggesting that a defect in the expression of specific cytokines is associated with severe liver disease.
Key words. Interferon; cytokines; interleukins; gene expression; transcription; autoimmune; disease.

Introduction

Mammalian cells produce and respond to a variety of secreted polypeptides or cytokines such as interferons (IFN), tumor necrosis factor (TNF), interleukins (IL), and growth factors which affect the proliferation, differentiation, and function of cells involved in numerous physiological processes[19, 44, 65, 67, 109, 123]. I have suggested previously that such intercellular messengers occupy a central position in a putative network of cytokine interactions which act to maintain homeostasis in normal tissues[126]. In this article I shall review the current evidence in support of this concept and suggest a model for the organization of the cytokine network which may help us to understand cytokine interactions in vivo. I shall also examine the situations in which uncontrolled production of a specific cytokine could lead to the development of disease.

If we are to understand how cytokines regulate cell physiology and maintain homeostasis in vivo we must first determine how the expression of specific cytokine genes is regulated in normal tissues. It is important to establish, for example, how cytokines such as interferons, TNF, and interleukins, which are induced proteins, are able to function as endogenous regulators of cell function in vivo when their production is usually subject to stringent control.

I shall not discuss the production of cytokines by cells in culture, except to illustrate interactions which may exist in vivo. Although in vitro systems are of undoubted value for the study of cytokines, caution is required when extrapolating from such simplified and by definition artificial systems to the complexity of the whole animal. Although there are, for example, a number of reports of

so-called spontaneous production of interferons and other cytokines in cells in culture [19, 126, 127, 137], it is often difficult to be sure that factors such as the in vitro manipulation of animal cells per se or the use of medium supplements such as serum do not act as inducers in such systems. Thus, IFN-β activity has been detected in several normal mouse tissues in organ culture but not in homogenates of fresh tissue from the same mice [115]. Low levels of IFN-β mRNA have also been detected in murine macrophages in culture in the apparent absence of induction [35]. Cultivation of human peripheral blood leukocytes in vitro has also been reported to induce the production of IL-6 mRNA in these cells [137].

The expression of interferon genes in normal tissues

Interferons are secreted proteins which modulate the expression of a number of cellular genes [31, 104] and exhibit pleiotropic biological activities including the establishment of an antiviral state, inhibition of cell multiplication, regulation of differentiation, and modulation of the immune system [19]. The production of interferon is usually considered to be subject to stringent control with significant quantities of interferon being produced only after induction [107, 145]. Thus, although human cells contain numerous gene loci encoding potentially functional interferons [51, 146] the expression of these genes is usually repressed. Induction leads to the activation of one or more interferon genes with transient transcription of the corresponding mRNA and synthesis of the interferon protein. There is evidence to suggest, however, that certain interferons may be produced in vivo under conditions other than virus infection and that this endogenous interferon may play an important role in regulating such important physiologic processes as fetal development and hematopoiesis [16, 75, 154]. Thus, an interferon-α-like substance has been detected in the plasma of healthy individuals [121], in the amniotic fluid of pregnant women [16, 75], in fetal organs [16], in human placenta [16, 29], in placental blood [16], in normal bone marrow sera [154], in the proliferative compartment of normal epidermis [150], and in human plasma following exercise [139]. Support for a possible role of interferons in pregnancy comes from the recent findings that the embryonic secretory protein of sheep, ovine trophoblast protein-1 (OTP-1), which is implicated in signalling maternal recognition of pregnancy, shares significant sequence homology with bovine alpha class II interferons [17, 59], and that human IFN-α-2 mimics the effects of OTP-1 on ovine endometrial cells in culture [117]. It has been suggested that ovine trophoblast proteins may represent interferons of specialized function [59].

The continuous presence of low levels of an interferon-like substance in the tissues of normal individuals could provide an important host defence against virus infection. The presence of low levels of interferon in vivo could explain, for example, the long-recognized resistance of freshly explanted human peripheral blood monocytes to virus infection and the decay of this antiviral state with time in culture [38]. The results of studies with experimental animals also suggest that interferon is present in the organs of normal mice [129], and that this interferon may play a role in host defence against both virus infection and neoplastic cells. Thus, explanted peritoneal macrophages from adult mice were found to be non-permissive for virus replication, whereas macrophages from mice pretreated with antibody to mouse IFN-α/β were permissive [7]. Treatment of normal immunocompetent mice with antibody to mouse IFN-α/β also increased the growth and invasiveness of a variety of murine tumors [39]. Significant levels of the interferon-induced proteins 2–5 oligo A synthetase [32, 46], p67K kinase [32], and MX [28, 41] have also been detected in the organs of normal uninfected mice which again suggests that interferon is produced continuously in vivo. Although there is considerable evidence for the presence of low levels of interferon in vivo, interferon activity cannot be readily detected in the tissues of experimental animals. This could be due to the presence of auto-antibodies to both IFN-α and IFN-β, which have been detected in a number of inbred strains of mice and rats [20–22].

A basis for an understanding of these different observations has come from recent studies in which the expression of interferon genes in vivo was analyzed by a modified S_1 mapping procedure capable of detecting very low levels of RNA transcripts specific for an individual interferon gene in extracts of normal tissue [130]. The use of these techniques has shown that a specific pattern of expression of interferon genes occurs in the organs of normal individuals and that this pattern differs from that seen following induction by either viruses or growth factors. Thus, in normal human spleen for example, IFN α_1 and α_2 are the only transcripts detected in the absence of induction. No IFN α_4, α_5, α_6, α_7, α_8, α_{14}, or β transcripts were detected in any of the samples tested [130]. This is in contrast to the presence of IFN α_1, α_2, and β_1 in human peripheral blood leukocytes or spleen cells following virus induction [130], and the presence of predominately IFN-β in cells following induction by lipopolysaccaride [6], platelet-derived growth factor (PDGF) [155], or colony stimulating factor (CSF) [109]. The absence of IFN-β_1 transcripts from the tissues of normal individuals cannot be attributed either to an inherent weakness of the IFN-β_1 promoter, since IFN-β is expressed to high levels in many different cells following induction [56], or to a lower sensitivity of detection, as 0.0003 fmoles of specific IFN-β_1 RNA can be detected under the experimental conditions used [130]. The detection of a characteristic pattern of expression of interferon genes in all the samples tested, together with the low number of transcripts produced per cell ($\geq$ 0.04 copies), argue against these results simply reflecting opportunistic virus infection, and suggest that specific interferon genes are transcribed in vivo in the apparent absence of induction.

The expression of cytokine genes in normal tissues

A novel cytokine variously designated IFN-β-2[82,152,153], 26KDa protein[48], B-cell stimulatory factor 2 (BSF-2)[54,55], hybridoma-plasmacytoma growth factor[142], colony-stimulating factor 309[33], hepatocyte stimulating factor[4], or interleukin-6 (IL-6)[105], has recently been shown to be produced by numerous types of human cells[68,69,82,105,120,152,153]. The expression of the IL-6 gene appears to be regulated in vivo in a manner quite different to that of the interferon genes. Thus, we have shown that the IL-6 gene is transcribed at relatively high levels in the organs of normal individuals[128]. IL-6 mRNA was present in human spleen at concentrations ranging from 0.6 to 16 copies per cell. Similar levels of IL-6 messenger RNA were also detected in liver, kidney, and peripheral blood leukocytes from normal individuals. This is in marked contrast to the absence of detectable IFN-β-1 transcripts (< 0.0003 copies/cell) in the same samples of human tissue. These results emphasize the marked difference in the regulation of the IL-6 gene and that of interferon α, β, or γ genes which are either not expressed at all or are expressed at levels some two hundred to two thousand times lower in the tissues of normal individuals. Interferon α, β, and γ genes are in fact regulated in vivo with a stringency comparable to that of proteins characteristic of a differentiated tissue[49,50,57,133].

Samples of tissue from normal individuals which contained high concentrations of IL-6 mRNA were also found to contain high levels of TNF, and IL-1 mRNA. As both TNF and IL-1 have been shown to be potent inducers of IL-6 in vitro[18,68,69,120] this raises the possibility that the presence of high levels of mRNA of these genes in normal tissues results from their continued induction by these or other cytokines.

The continued presence of relatively high levels of IL-1α, IL-1β, IL-6, and TNF mRNA in the tissues of normal individuals provides evidence for the concept that homeostasis is maintained by the concerted action of a number of cytokines, many of which have multiple activities. The presence of cytokines such as IL-1, IL-6, and TNF in normal tissues could, for example, constitute a primary means of regulating cell proliferation in vivo as all of these cytokines can act as growth factors for certain cells while inhibiting the multiplication of others[18,68,69,83,105,120,152,153].

Cytokines such as IL-6 may also function as negative regulators of the mitogenic activity of growth factors such as PDGF[68,69] and other cytokines such as TNF[68,69]. The possible ramifications of the action of IL-6 are considerable as the mitogenic action of PDGF, for example, is mediated by the induction of a number of cellular genes including the proto-oncogenes c-myc and c-fos[114].

It is becoming increasingly clear that soluble factors such as the lymphokines and other cytokines play a determining role in the regulation of the immune system. Soluble factors are involved in such processes as lymphocyte activation and differentiation and in the coordinated action of helper T-cells and B-cells which leads to the production of antibody[100]. The importance of IL-1 and IL-6 in both B-cell maturation[54,55] and T-cell activation[33] suggests that these cytokines may also play an essential role in the regulation of both humoral and cellular immunity in normal tissues. IL-1, IL-6, and TNF also play an important role in the inflammatory response, both in the local reaction involving degranulation of mast cells and an increase in vascular permeability[81], and in the systemic induction of fever[25,26] and release of acute phase proteins[4,9,40].

Although IL-6 shares certain properties common to interferons[82,152,153] it remains a point of contention whether IL-6 posseses antiviral activity and can thus be considered an interferon[10,105,110,120]. There is, nevertheless, considerable evidence to suggest that antiviral activity is detected in situations where TNF, IL-1, and IL-6 are present[48,55,86,141,149,152]. Thus, the detection of high levels of expression of these cytokines in the tissues of normal individuals together with the importance of IL-1, and IL-6 in B-cell maturation raises the possibility that the continued presence of these cytokines in vivo may play an important role in host defense against virus infection both in the initial antiviral response and in the later development of antibody.

Regulation of the cytokine network in vivo

The study of the regulation of the complex pattern of actions and interactions which comprise the cytokine network in normal tissues is a prerequisite for an understanding of how multifunctional cytokines which can have apparently opposite effects on different cells can exert a precise effect in a particular system. I should like to suggest a possible way in which the cytokine network may be organized which may help to explain the seemingly bewildering array of cytokine interactions in normal tissues. If one considers cytokines as intercellular regulators of gene expression then one can define three levels at which cytokines could act; at the level of cytokine production, at the level of specific cell surface receptors, and at the level of transcription. Coordinate regulation of cytokine action at these three levels would thus allow a very fine degree of control of cellular function to be attained.

Let us consider the first level of control: regulation of the level of production of a cytokine. As discussed above, recent results from our laboratory have shown that the genes of cytokines such as TNF, IL-1, and IL-6 are expressed at relatively high levels in the tissues of normal individuals[128]. Certain interferon genes are also expressed in normal tissues albeit at very much lower levels[130]. Numerous studies have shown that cytokines can influence the production of other cytokines in a highly

complex manner. Thus both TNF and IL-1 are potent inducers of IL-6 [18, 68, 69, 142]. TNF has also been reported to induce the production of IL-1 [71, 76, 90] and IL-1 to induce TNF [101]. IL-1 can also stimulate its own production [27, 144]. This is of course only part of the picture, as the production of each of these cytokines influences the production of other cytokines and is in turn influenced by yet other cytokines. IFN-β, for example, which is often produced together with IL-6 can also stimulate the production of both TNF and IL-1 [14, 52, 118] and both TNF and IL-1 can in turn induce the production of IFN-β [141]. The production of cytokines may also be subject to yet further levels of control. There is evidence to suggest, for example, that the production of TNF, IL-1, and IL-6 may be regulated by neuropeptides released from sensory neurons at the site of tissue injury or inflammation [78].

It is clear that factors such as TNF, IL-1, and IL-6 are not the product of a single cell type as was once thought but are in fact produced by cells of many different lineages [96], and high levels of these and other cytokines have been detected in the spleen, liver, kidney, and peripheral blood leukocytes of normal individuals [128]. It seems likely, therefore, that the local concentration of a cytokine and consequently the effect exerted by that cytokine can be modulated by the presence of other cytokines.

The second level of regulation would be via receptor modulation. Binding of a cytokine to its high affinity receptor results in the transfer of information from the cell surface to the nucleus via a process which in the case of some growth factors or other cytokines may involve activation of protein kinase C [136]. The localized production and action of a cytokine could provide a means of attaining specificity if its action was a function of the target tissue. The sensitivity of the cells of a particular tissue to a cytokine could be determined by the modulation of the expression of specific cell surface receptors. Receptor expression may be influenced both by the presence of the specific cytokine as well as structurally unrelated cytokines. TNF, for example, modulates the expression of its own specific high affinity receptor which in turn is modulated by the presence of IFN-γ [2, 134, 135]. Under physiological conditions receptor modulation may be a primary means of regulating the action of cytokines such as TNF, IL-1, and IL-6, the RNA transcripts of which are present constantly in the tissues of normal individuals [128].

Cytokines may function in normal tissues both in an autocrine manner, that is on the producing cells themselves via specific cell surface receptors, or in a paracrine manner on neighboring cells. Interferon produced by mature mononuclear cells in response to CSF-1 has been shown both to decrease the number of cell surface receptors for CSF-1 on mature macrophages and to inhibit the growth response of committed precursor cells to CSF-1 [87, 88] illustrating how the same substance can exert a different effect on different cells even within the same tissue. The activity of a cytokine such as IL-6 which is expressed at high levels in both lymphoid and non-lymphoid tissues may be a function of its tissue distribution. The ability of IL-6 to induce B-cell maturation in the absence of an effect on cell multiplication [54, 55], for example, may be important in certain lymphoid tissues while its activity as a growth factor [142] may predominate in other tissues. Cytokines can exhibit either synergistic or antagonistic interactions which may in part be receptor-mediated. TNF and IFN-γ, for example, exhibit synergism in inhibiting cell proliferation and tumor growth [3, 30], in the induction of MHC class II antigens [15], and in their antiviral action [149]. In contrast the mitogenic action of TNF in human fibroblasts is antagonized by the presence of IFN-β [138]. Under physiological conditions the activity expressed by a particular cytokine may be determined, therefore, by tissue-specific interactions which may in turn be influenced by other cytokines.

The third and ultimate level of regulation would be through transcriptional contol. In higher eukaryotes specific genes are regulated by cis-acting DNA sequences which interact with specific transcription factors [79, 125]. Although certain general transcription factors are required for the transcription of all genes transcribed by RNA polymerase II [79, 125], other promoter-specific transcription factors recognize specific nucleotide sequences present in the promoters of only certain genes [79, 125]. A number of protein-protein interactions are also required for accurate and controlled initiation of transcription [79, 125]. The expression of a wide variety of genes has been shown to be controlled by both positive and negative regulatory elements which respond to nuclear transcription factors induced or activated by extracellular factors [79, 125]. Several cellular genes have been shown, for example, to contain interferon-responsive elements in their 5' flanking region [79, 125]. Interferons can also activate specific transcription factors [87]. I suggest therefore, that the activites exhibited by a particular cytokine may be determined by the complement of transcriptional regulatory proteins induced or activated by the cytokine. This complement of transcriptional proteins would be in part common with that induced by other unrelated cytokines, which could explain the often overlapping activities of different cytokines.

Cytokines and disease

In view of the importance of cytokines in the regulation of numerous physiological processes it would seem likely that uncontrolled production of a particular cytokine could lead to the development of disease. Abnormal production of interferon α has been detected in patients with certain autoimmune or immune deficiency diseases such as systemic lupus erythematosus [58, 64, 99], Behcet's disease [58], aplastic anemia [154] and the acquired immune deficiency syndrome (AIDS) [24]. Interferon γ has also been

detected in the lesions of patients with Behcet's disease[94], Sjogren's syndrome[116], aplastic anemia[53], and in the lesions of patients with certain inflammatory diseases such as rheumatoid arthritis[12], pulmonary sarcoidosis[112], or proliferative disorders such as psoriasis[11]. Both IFN-α, and IFN-γ have also been detected in patients with diabetes mellitus[131]. Furthermore, the production of interferon during the course of a virus infection may be responsible for many of the symptoms such as fever, fatigue, and leukopenia commonly associated with virus infection[119]. Interferons may also play a role in the pathology of certain virus diseases such as congenital rubella and progressive familial encephalopathy[73,74] and may even contribute to the fatality of virus diseases such as Argentine hemorrhagic fever[76].

The production of TNF is associated with certain disease states such as the severe weight loss or cachexia which accompanies chronic parasitic or bacterial diseases and cancer[98], septic shock[36,132], aplastic anemia[53], and acute graft-versus-host disease[103]. IL-1 which has multiple biological activities including that of an endogenous pyrogen[25] and inducer of acute phase proteins[9] is produced by a variety of cell types in response to infection or tissue injury[138]. IL-1 is also produced during the course of inflammatory diseases such as rheumatoid arthritis where it is thought to play an important role in both the development of the local inflammatory reaction and in the degenerative process through the stimulation of the production of factors such as procollagenase by rheumatoid synovial fibroblasts[84,143]. High levels of both IL-2 and IL-2 receptor transcripts have also been detected in mononuclear cells of rheumatoid joint lesions[13].

It is well established that the proliferation of certain tumors is dependent on the presence of growth factors or cytokines often produced in an autocrine manner by the tumor cells themselves[123]. The B-cell stimulatory factor and plasmacytoma growth factor IL-6, for example, is required for the proliferation of human multiple myelomas[62,66]. It remains unclear, however, whether IL-6 is produced principally by the tumor cells themselves[62] or by adherent cells of the local bone marrow environment[66]. The osteoclastic bone destruction and hypercalcemia observed in patients with myeloma appears to be due largely to the production of TNF-β, otherwise known as lymphotoxin[34], possibly acting in conjunction with other bone absorbing cytokines such as TNF-α, IL-1, and IL-6[8,37,66]. Transforming growth factor α (TGF-α) is a secreted polypeptide structurally related to epidermal growth factor (EGF) which binds to the EGF receptor[23]. TGF-α is produced in normal tissues such as the pituitary, and the brain, and at the site of tissue injury where it is thought to play an important role in the processes of healing[108]. TGF-α is also secreted by a number of tumors and, in conjunction with TGF-β which binds to its own specific receptor[23], is thought to play a role in the process of malignant transformation[23,67]. It is unclear, however, whether tumor cells produce more TGF-α than normal cells or are more responsive to the action of TGF-α[23].

Support for the concept that the abnormal production of cytokines is not just a marker of disease, but can also cause disease, comes from animal studies which show that the endogenous production of interferon during the course of a virus infection, or the production of TNF during a bacterial infection, can cause disease and death as can the administration of large quantities of exogenous interferons, or TNF. Thus the use of specific antibody to mouse interferon has shown that production of IFN-α/β causes the acute liver disease and contributes to the later development of glomerulonephritis in newborn mice infected with lymphocytic choriomeningitis (LCM) virus[43,111,112] and is a cause of death in adult mice inoculated intracerebrally with LCM virus[100]. Similarly, the use of antibody to TNF has shown that production of this cytokine is responsible for septic shock during lethal bacteremia in baboons[132]. Treatment of newborn mice with exogenous IFN-α/β or TNF has also been shown to induce liver necrosis and death[45,47]. Animals which survive interferon treatment also develop glomerulonephritis later in life whereas animals treated with TNF do not[47]. The use of antibody to mouse IFN-γ has shown that production of IFN-γ is involved in the development of glomerulonephritis in NZB/NZW F1 mice[60]. Treatment of NZB mice with exogenous IFN-α/β, or IFN-γ has also been shown to accelerate the onset of glomerulonephritis in these animals[1,60]. IL-1 has also been implicated in the development of glomerulonephritis in rats[147]. In view of the complexity of the cytokine network in vivo it is not surprising that several distinct cytokines may be involved in the physiopathology of a particular disease.

Animal studies also provide evidence that a defect in the production of a particular cytokine may also be involved in the pathogenesis of certain diseases. Thus (NZB × NZW) F_1 hybrid mice which develop severe glomerulonephritis have been shown to produce less TNF_α (but not IL-1) than parental NZB or normal Balb/c mice. Treatment of F_1 hybrid mice with recombinant TNF_α has also been shown to delay the onset of nephritis in these animals[61]. The inhibition of growth and perturbation of glucose metabolism in newborn mice infected with LCM virus has been reported to be due in part to a reduction in the production of growth hormone following virus infection rather than to a direct cytocidal effect of the virus[97].

Several studies suggest that a defect in the production of a specific cytokine may also be associated with the pathogenesis of certain human diseases. The defect in cell-mediated immunity in patients with lepromatous leprosy, for example, has been shown to be associated with a lack of IFN-γ production[93]. IFN-γ production is also defective in patients with AIDS[90], and in patients with X-linked lymphoproliferative syndrome[151]. There is also some evidence to suggest that IFN-γ production may be

impaired in patients with multiple sclerosis[92]. Recent studies from our laboratory have shown that the level of expression of IL-6, also known as hepatocyte stimulating factor[4], but not IFN-α, is markedly reduced in the liver of patients with primary biliary cirrhosis compared with the levels present in normal human liver (Tovey et al; unpublished results), raising the possibility that reduced expression of IL-6 or other cytokines is involved in the pathogenesis of this disease.

The potential clinical benefit of the use of cytokines in both adjuvant and replacement therapy in life-threatening situations is sufficient to outweigh the potential danger of the use of highly active biological substances of unknown mechanism of action. Both interferons and IL-2, for example, have been shown to inhibit the growth of liver and lung metastases in experimental animals[42, 70, 83], and interferons have been used successfully in the treatment of several viral and neoplastic diseases of man[85, 122, 124]. The toxicity associated with cytokine therapy can be considerable, however, as has become apparent with the recent use of IL-2, and TNF in the treatment of cancer[95, 148]. The use of antibody to a particular cytokine may also find application in the treatment of certain acute diseases. Antibody to TNF, for example, has been shown to protect baboons against lethal bacterial septic shock[132]. The widespread use of cytokines in the therapy of human disease will, however, have to await a better understanding of both the role of cytokines in the regulation of normal physiological processes as well as the nature of the dysfunction of cytokine production involved in the pathogenesis of human disease.

Conclusions

The concept that homeostasis is maintained in normal tissues by the concerted action of a network of cytokines is now supported by a considerable body of evidence. We are, however, only starting to unravel the myriad actions of the numerous multifunctional cytokines present under physiological conditions, and an understanding of the finer details of cytokine interaction in vivo may well elude us for some time to come. The elucidation of cytokine interactions in normal tissues will allow both a better understanding of how homeostasis is maintained at the cellular level, and of how the immune system functions under physiological conditions. Such information will also open the way for determining the role of alterations in the production of specific cytokines in the pathogenesis of diseases involving immune dysfunction, which has important implications for the therapy of human disease.

Acknowledgments. This work was supported in part by grants from the Association pour la Recherche sur le Cancer à Villejuif, the Centre National de la Recherche Scientifique, the Fondation pour la Recherche Médicale, the Commission des Communautés Européennes (St.2J.0317 and St.2A.0003F), and the Institut National de la Santé et de la Recherche Médicale (CRE 881018).

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0014-4754/89/060526-10$1.50 + 0.20/0

Interferons and indoleamine 2,3-dioxygenase: Role in antimicrobial and antitumor effects

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Summary. Indoleamine 2,3-dioxygenase (IDO) is an interferon (IFN)-induced protein that initiates the metabolism of tryptophan along the kynurenine pathway. Although IDO can be induced by IFN-γ in many cell types, only mononuclear phagocytes have been shown to be induced to decyclize tryptophan by all three IFN classes. Since tryptophan is an essential amino acid necessary for a variety of metabolic processes, depletion of available tryptophan may be an important mechanism for control of rapidly-dividing microbial pathogens and tumors. The purpose of this review is to present evidence that documents the effects of IFN-induced IDO on prokaryotic and eukaryotic pathogens, as well as on a variety of tumor cell lines.
Key words. Tryptophan; macrophage; monocyte; interleukin-2; cancer; *Chlamydia; Toxoplasma.*

Introduction

Since its initial characterization as a viral interfering substance[22], significant advances in describing the diverse effects of the interferon system (IFN) have occurred. No longer is IFN considered to affect only viruses; immunomodulatory, antimicrobial, and antitumor effects have been demonstrated using IFNs. Although the mechanisms of action for many of these effects have yet to be precisely determined, a number of proteins have been shown to be induced by interferons[58]. Presumably, as research continues, specific functions will be attributed to these proteins, and specific mechanisms of action will become better defined.

One IFN-induced protein for which a function has been demonstrated is indoleamine 2,3 dioxygenase [IDO; indoleamine:oxygen 2,3-oxidoreductase (decyclizing)]. IDO has been purified from both animal and human tissues, and has been characterized as a cytoplasmic, heme-containing, monomeric protein of approximately 40,000 molecular weight[36,49,63]. Unlike the liver-specific enzyme tryptophan 2,3-dioxygenase, a 168,000 molecular weight tetrameric enzyme with four heme-chromophores at its active site[19,24], IDO is ubiquitously distributed in normal[49,63,67] or malignant tissues[64,68], mononuclear phagocytes[12,13,34,59] and neoplastic cell lines[16,35,51,60]. IDO can be induced by cancer[68], viral infection[71], bacterial lipopolysaccharides (LPS)[13,65,69], IFN[9,11,12,16,34,35,51,52,59,60,64,66,70] and interleukin-2 (IL-2)[5,12,13]. In each system studied, induction of IDO has been shown to result in substantially increased tryptophan metabolism. Among the indoleamines against which IDO has been shown to possess activity are included D- and L-tryptophan, D- and L-5-hydroxytryptophan, tryptamine and serotonin[49]. However, IDO has been shown to be most active against the essential amino acid L-tryptophan. Its enzymatic activity involves the decyclization of tryptophan to *N*-formylkynurenine by oxidative cleavage of the pyrrole ring. Apparently, both molecular oxygen and superoxide anion can participate as the second substrate for this reaction[36,50,53]. The biological electron donors for the heme iron of IDO are proposed to be reduced flavin and pyridine nucleotides[36], as well as superoxide anion formed by coupling electrons from tetrahydrobiopterin[37], flavoenzymes, xanthine oxidase or glutathione reductase[20,21,53].

Although the exact physiologic function of IDO has not yet been completely defined, experimental evidence suggests that it may be involved in regulation of cellular growth and proliferation. Since tryptophan concentration either in the individual cell or in the whole organism is important for several metabolic processes, its regulation by IDO may have pleiotropic effects. Tryptophan is an essential amino acid and the least abundant of the amino acids required for mammalian cellular integrity. Its availability affects protein synthesis as well as protein degradation, genome replication, and organismal growth[2,7,15,27,28]. Restriction of available tryptophan due to degradation by IDO could lead to a condition in which cells become starved for tryptophan. Such a situation would more severely affect rapidly dividing cells such as microbial pathogens and tumor cells. However,